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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 2 July 2003 with an application for Letters Patent number 526804 made by RICHARD HAMILTON ARCHER and DEREK ROBIN HAISMAN.

Dated 2 August 2004.

Neville Harris
Commissioner of Patents, Trade Marks and Designs

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NEW ZEALAND

330 High Street, Lower Hutt and 17 Toop Street, Seaview
PO Box 30687, Lower Hutt, New Zealand or DX SX 11129, Wellington

NEW ZEALAND PATENTS ACT 1953

PROVISIONAL SPECIFICATION

PROCESS FOR PRODUCING A MODIFIED DAIRY PRODUCT

We, RICHARD HAMILTON ARCHER, a New Zealand citizen of 5 Hillgrove Place, Palmerston North, New Zealand and DEREK ROBIN HAISMAN, a British citizen of 20 Lees Road, R D 5, Feilding, New Zealand, do hereby declare this invention to be described in the following statement:

TECHNICAL FIELD

The present invention relates to production of dairy products containing bacterial exopolysaccharides.

BACKGROUND ART

Bacterial exopolysaccharides are well known not only for their role in bacterial structure but also as food ingredients. In particular they have been found useful as thickeners and stabilisers in food products.

For example Xanthomonas campestris is well known for the production of an exopolysaccharide which may be isolated to form xanthan gum widely used in the food and pharmaceutical industries. Xanthan gum is used in dairy products such as ice cream, yoghurt, cheese spread and cream cheese.

Generally when exopolysaccharide such as xanthan gum is introduced into dairy products, the exopolysaccharide is an extract or purified polysaccharide separated out from a ferment. For example commercial xanthan gum is produced by a process involving treatment with heat and extraction with isopropanol to separate the xanthan gum from the other components of the ferment.

Another approach to increasing the exopolysaccharide content of a dairy product is to include live microorganisms in the product.

Both of these approaches have disadvantages. The processing steps required for separation of the exopolysaccharide from a fermentation mixture mean that such exopolysaccharide preparations are expensive and may contain traces of precipitating agent. The addition of live microorganisms creates problems in controlling the amount and quality of the exopolysaccharide added. Also addition of live microorganisms may lead to incorporation of byproducts of the microorganism into the dairy product.

It is an object of the present invention to provide a simple controlled process for adding exopolysaccharide to a dairy product, or at least to provide the public with a useful choice.

DISCLOSURE OF THE INVENTION

In one aspect, the invention provides a process for the modifying of a dairy product comprising mixing into the product a killed ferment of an exopolysaccharide-producing-microorganism without isolating the exopolysaccharide from the ferment.

The killed ferment may be directly mixed into the product. Alternatively the ferment may be mixed into an ingredient used in making the product.

Preferably the killed ferment is a ferment prepared using a lactose-rich medium and an exopolysaccharide-producing-microorganism. In some cases where the microorganism does not hydrolyse lactose, the ferment will require addition of a lactase or galactosidase enzyme or an organism which produces an enzyme which hydrolyses lactose. A lactose-rich medium is a medium containing more than 0.5% (w/v) lactose, preferably more than 1.0% (w/v).

Preferably the lactose-rich medium is a fraction of milk such as skim milk or buttermilk or whey or serum or mother liquor; or raffinate or flowthrough derived from milk or skim milk or buttermilk or whey or serum or mother liquor or permeate; or permeate derived from milk or skim milk or buttermilk or whey or serum or mother liquor or raffinate or flowthrough.

Preferably the microorganism is a food-acceptable microorganism.

Preferably the lactose-rich medium comprises a dairy permeate.

Preferably the dairy permeate is a milk permeate or a whey permeate.

When a dairy permeate is used the invention provides the advantage that a readily available by-product of a dairy factory is used without the disadvantage of having to further process and transport it to other sites. In addition in this aspect the invention allows combinations of dairy streams generated within the same plant to be used to provide product with enhanced value.

Preferred microorganisms for use in the invention are *Xanthomonas campestris*, *Sphingomonas paucimobilis* and lactic acid bacteria.

Currently particularly preferred are *Xanthomonas campestris* and *Sphingomonas paucimobilis* which produce exopolysaccharides known as xanthan and gellan respectively.

Lactic acid bacteria useful in process of the invention include *Lactobacillus delbrueckii* ssp *bulgaricus*; *Lactococcus lactis* ssp *cremoris*; *Lactococcus lactis* ssp *lactis*; *Streptococcus salivarius* ssp *thermophilus*; *Lactobacillus casei* ssp *casei*; *Leuconostoc mesenteroides*; *Lactobacillus helviticus; Lactobacillus reuteri; Lactobacillus rhamnosus; Lactobacillus plantarum; Lactobacillus sakei.*

Other microorganisms useful in the process are microorganisms selected from the genera *Pseudomonas, Zooglea, Rhizobium, Agrobacterium, Klebsiella, Acetobacter, Alcaligenes, Leuconostoc, Propionibacterium, Microcystus, Azotobacter, Acinetobacter* and *Enterobacter.*

Generally, the fermentation conditions are selected to maximise the yield and quality of exopolysaccharide.

Typically the incubation is conducted at a temperature of 10°C to 60°C, more commonly 20-35°C.

The ferment may be killed by heating. The heating conditions required vary depending on the susceptibility of the microorganism to heat. Generally heating to a temperature in the range 50°C to 100°C for 1-5 minutes is preferred.

Other methods of killing the ferment compatible with food use may also be used – for example by gamma irradiation, redox shift, pH excursion, osmotic shock, electropulse, ultra high pressure, lysing enzyme, high-shear lysis, electron beam radiation, ultraviolet radiation or other types of electromagnetic radiation, or combinations of these.

In the methods of the invention, the exopolysaccharide is not isolated. However it is contemplated that the ferments may undergo other types of treatment such as desalting or treatment to remove flavour

components. Desalting may be carried out using ultrafiltration, nanofiltration, electrodialysis or ion exchange. Flavour components may be removed by contacting the ferment with flavour-adsorbing materials, for example activated charcoal. Unwanted particulates may be removed by centrifugation or filtration.

In a preferred embodiment of the invention, the microorganism is added to a dairy permeate medium with added nutrients for the microoganism such as appropriate salts, a supplementary nitrogen source, a yeast extract and exopolysaccharide stimulating factors. The mixture is then typically incubated for 16-240 hours, generally 60-120 hours. The exopolysaccharide concentration may be determined. At this stage the ferment may be heated and spray dried and subsequently added to a dairy product or an ingredient of a dairy product or ingredient. Alternatively the ferment may be killed and mixed directly with a dairy product.

The methods of the invention are distinguishable over those generally used in the prior art in that there is no requirement for separation of the exopolysaccharide from the medium. In addition to saving on costs, this avoids any harsh extraction process which may modify the properties of the exopolysaccharide. The process is distinguishable and advantageous over those involving incorporating live organisms in that the amount and quality of exopolysaccharide added can be more readily controlled, for example the exopolysaccharide concentration may be measured and the incubation conditions such as the carbon:nitrogen ratio readily adjusted and controlled. In addition the production of byproducts may be controlled.

The process of the invention is useful for providing thickened and stabilised dairy products and for producing ingredients useful in preparing such thickened and stabilised dairy products. For example the invention may be used for preparing an exopolysaccharide-containing milk. This may be consumed directly as a thickened milk. This product may also be dried for subsequent reconstitution. Additionally or alternatively it may be used to prepare other types of dairy products such as an ice cream, yoghurt or dairy desserts. Such products have the advantage of having increased yields by addition of relatively cheap solids and water.

In addition the invention may be used in cheese manufacture.

Exopolysaccharide-containing killed ferment may be added to milk or milk protein concentrate (MPC) to be used in cheese manufacture. Use of such modified milk or milk protein concentrate in conventional cheese making process and in which a proteolytic enzyme is added to cheese milk to produce a curd has the advantage of minimising loss of whey proteins during the cheese making process.

The process of the invention may also be used to prepare processed cheese and in other types of cheese making process such as the process of US Patent 6,177,118 and other processes for making cheese without using enzymatic hydrolysis.

Thickened milk, the modified MPC or the cheese prepared by the method of the invention can be used in conventional methods of preparing processed cheese or cheese sauces and other cheese-based products.

In another aspect the invention provides a process for the modification of a milk protein concentrate comprising adding to the concentrate a killed ferment of exopolysaccharide-producing-microorganism without separating the exopolysaccharide from the other components of the ferment. Such milk protein concentrates may be used in cheese extension. When such a milk protein concentrate is added to the milk to be used in a cheese making process, it provides the advantage of a high yield of cheese as do milk protein concentrates generally. In addition there is the further advantage of having improved retention of whey proteins in the cheese. Also the presence of the exopolysaccharide modifies the consistency of the cheese in a manner which is desirable in some cheese types.

In other aspects the invention provides products of the processes of the invention.

EXAMPLES

The following examples further illustrate practice in the invention. Where the basis of percentages has been omitted, it is w/w for solids, w/v for solids in liquids and v/v for liquids.

Example 1.

Sphingomonas paucimobilis (ATCC 31461) was cultured on milk permeate in a number of shake flasks to produce viscous broths containing the anionic polysaccharide known as gellan.
The inoculum of Sphingomonas paucimobilis was maintained in trypticase soy broth, and introduced into the fermentation medium at 2.5% (v/v). The milk permeate medium (milk permeate containing 0.1% yeast extract (Difco)) was free-steamed at 100°C for 10 min prior to inoculation of the culture and subsequent fermentation. The pH of the media was not adjusted prior to heating. Each 250 ml shake flask contained 25 ml of milk permeate and was incubated on an orbital shaker for 96 hours at 30°C.

After incubation the flasks were analysed for polysaccharide as follows. An aliquot of the ferment (1 g) was weighed into a centrifuge tube, heated to boiling for 10 minutes, and then mixed with 2 ml of ethanol (99%). The floccular precipitate was centrifuged down at 12,000 RCF, and the supernatant discarded. The pellet was redissolved in 1.5 ml water, and re-precipitated with 3.5 ml of 99% ethanol. After re-centrifugation, the pellet was dissolved in water and made up to 200 ml. Aliquots (1 ml) of this solution were mixed with 1 ml of phenol solution (5%, freshly prepared). Concentrated sulphuric acid (5 ml) was added, and the absorbance of the solution measured at 485nm after standing for 30 minutes. The polysaccharide concentration, in glucose equivalents, was calculated from the absorbance, after subtraction of the blank value from a water sample, by reference to a calibration plot constructed from assays using pure glucose.

Twenty flasks were combined to make a total of 3.7 g of polysaccharide. This was diluted to 750 ml, with reverse osmosis (RO) water and intimately mixed with 750 ml of reconstituted whole milk (20% solids in RO water). The milk was heated to 50°C and then pumped into a spray drier, with an inlet temperature of 180°C and an outlet temperature of 90°C. A free-flowing powder was collected, and the gellan content was determined by the same method as before after first hydrolysing the milk protein by incubation with a protease (Flavourzyme, Novozyme). The gellan content was found to be 2.5% on the milk solids.

The powder was dissolved in RO water to make solutions of 10, 15 and 20% solids. Control samples at the same concentration were prepared with spray dried whole milk powder without added polysaccharide. The viscosities of the different milks were measured at 25°C, using a capillary viscometer (Cannon-Fenske, Size 300). The powder containing the polysaccharide made a much thicker milk, as shown in Table 1.

Table 1. The viscosity of homogenised whole milks with and without added polysaccharide.

Solids level (%)	Viscosity (mPa.s)	
	Control	With 2.5% polysaccharide (on powder)
10	1.25	5.8
15	1.39	26.4
20	1.62	54.1

Example 2.

Xanthomonas campestris (ATCC 13951) was maintained on "YM" agar and reconstituted in "ISP" medium. It was inoculated (5% v/v) into a fermentation medium consisting of lactase-treated milk permeate (60% of the total volume), urea (0.10% w/v), K_2HPO_4 (0.20% w/v), and $MgSO_4.7H_2O$ (0.01% w/v). The urea and minerals were sterilized separately, and pooled with the hydrolysed milk permeate which had been steamed at 100 °C. The final medium pH was 7.0.

Fermentations were undertaken in shake-flasks (250 ml) in 100 ml volumes. The flasks were incubated for 96 hours at 28°C with agitation at 180 rpm on an orbital shaker. After this time the medium had become a viscous, pale yellow broth, with a slightly unpleasant odour.

Eight flasks were then combined and passed through a pressurized filter bed (18mm*125mm diameter) of granular activated carbon (Norit GAC 1240), which had been washed with 9 bed volumes of water. The viscosity of the broth decreased from 150 mPa.s to 126mPa.s on the first pass, as it mixed with the

water retained in the filter bed, but then remained constant through three further cycles through the bed. This was sufficient to decolourise and deodorise the broth.

After analysis (by the method in example 1) of the EPS content, 700g of broth, containing 4.9g of xanthan polysaccharide, was mixed with 485g milk protein concentrate (70% protein) in 2.2 litres of RO water. The mixture was homogenised with a Silverson overhead homogeniser for 5 minutes, passed through a sieve (300μm aperture), warmed to 50°C and then pumped to a spray-drier. The inlet temperature was 180°C, and the flow rate of the milk feed was adjusted to maintain the outlet temperature between 80 and 90°C.

A free flowing powder was collected and found to contain 1.056% xanthan polysaccharide, by the same analytical methods used in Example 1. This powder was used to make a soft white cheese, similar to the South American cheeses known as Panela or Queso Fresco. The basic composition of the cheese was 14 to 18% protein, 10 to 12% milk fat and 70 to 75% moisture. The cheese made was made by mixing a milk fat emulsion with milk protein concentrate, acidifying, adding salt and then incubating with rennet until the cheese was set.

Milk protein concentrate (20g) was dispersed in 480g of RO water at 50°C. Melted milk fat (500g) was added, and a coarse emulsion made with a Silverson homogeniser. This was then fully homogenised in a Rannie homogeniser at 70/50bar.

Two milks were then prepared, one with control spray dried milk protein concentrate, and one with the milk protein concentrate containing 1.056% xanthan polysaccharide.

The milk protein concentrate (170g) was dispersed in 494g RO water at 50°C in a pestle and mortar. The solution was stirred with a Heidolph RZR 50 stirrer for one hour, and then passed through a 300μm sieve.

Six different cheese milks were then prepared according to Table 2, by mixing the milk base, the fat emulsion, the water and lactic acid (2%) at 50°C, then adding the salts.

The temperature of the milks was then adjusted to just under 38°C, the rennet (Chymax) was added and then the milks were distributed into pottles and placed in an incubator at 38°C for 40 minutes to coagulate. The pottles were then transferred to a cool room at 4°C and cooled overnight.

Table 2. Formulation of the cheese milks

No	Control Milk Base (g)	Xanthan Milk Base (g)	Emulsion (g)	NaCl (g)	Calcium Lactate (g)	Lactic Acid Solution (g)	Water (g)	Rennet (µl)
1	-	170	50	3.0	0.375	30	-	40
2	-	140	50	3.0	0.375	30	30	40
3	85	85	50	3.0	0.375	30	-	40
4	70	70	50	3.0	0.375	30	30	40
5	170	-	50	3.0	0.375	30	-	40
6	140	-	50	3.0	0.375	30	30	40

The following day, the solids content, texture and whey loss from the cheeses were measured moisture was determined by oven-drying at 102°C overnight.

The texture of the cheeses was measured with a TA XT2 texture analyser (Stable Micro Systems, Surrey, England), and was conducted a day after samples were made. A scalpel was used to cut away the plastic pottle and 10mm cubes were cut out of the cheese. Measurements were conducted using the TA XT2 texture analyser at 5°C using two plates in which the cheese cubes were placed in the centre of the two plates and the force applied. The test speed was 5 mm/s, compressing the cube to 7mm. The test was carried out a minimum of four times. The results were plotted as force against time, and the area under the graph calculated as a measure of the firmness of the cheese.

For the measurement of free whey, the cheese was cut into 5mm cubes at 5°C and placed into a 50ml centrifuge tube with a cone bottom until a minimum of 5g had been added. Measurements were carried out in quadruplicate. The cheese was left in an incubator at 21°C for 3 hours then centrifuged (RCF 112) for 10 minutes. The whey was poured off and weighed. The centrifuge tube was then inverted at 30° for 2 minutes and the residual drained whey added to the weight. The whey loss was calculated as a percentage of the original weight of cheese.

The results for the six cheeses are shown in Table 3. The measured solids in the cheeses corresponded closely to the calculated values of 27.48 and 25.74% for the two levels of protein.

There was little difference in firmness between the samples, except for the cheese with the highest level of xanthan in the milk powder, and the highest level of protein. This cheese was slightly less firm than the others, matching the texture of the samples with the lower level of protein. This type of cheese is characteristically quite soft, and the difference was barely detectable when the cheeses were compared in the mouth.

There were much larger differences in whey loss between the cheeses. The cheese with the highest level of xanthan and protein lost significantly less whey than all the others, and clearly less milk protein concentrate containing this level of polysaccharide would be needed to make cheese of equivalent whey holding capacity to the control sample at this level of protein. The cheese made with 25.74% milk protein concentrate containing 1% polysaccharide was equivalent in whey holding capacity to the cheese with 27.48% protein concentrate. This represents a saving in milk powder of over 6%.

Table 3. Properties of Experimental Cheeses

Milk protein concentrate (% of cheese)	Xanthan polysaccharide (% of cheese)	Total solids (%)	Firmness N.s	Whey Loss (%)
17.57	0.186	27.57 (0.13)	3.66 (0.45)	7.19 (3.49)
17.57	0.154	28.12 (0.14)	5.06 (0.43)	15.64 (0.83)
17.57	0	28.18 (0.12)	5.37 (0.30)	20.01 (0.48)
14.54	0.093	24.42 (0.1)	3.58 (0.14)	14.68 (2.35)
14.54	0.077	25.16(0.21)	3.74 (0.21)	20.51 (1.88)
14.54	0	25.37 (0.23)	3.95 (0.22)	22.99 (0.45)

Note: Standard deviations in brackets

The above Examples are illustrations of practice of the invention. It will be appreciated by those skilled in the art that the invention can be carried out with numerous modifications and variations. For example the type of microorganism used may be varied. The microorganisms used may be producers of different exopolysaccharides. The fermentation media, carbon source and times and temperatures may be varied. The killed ferments may be added to different types of milk products.